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MORPHOLOGICAL CHANGES OF THE LIVER, KIDNEYS, AND ADRENAL GLANDS OF EXPERIMENTAL ANIMALS AFTER LONG TERM FEEDING WITH ROUNDUP READY GENETICALLY MODIFIED SOYA

Summary. *For 485 days, pigs were fed 15-20% raw protein from thermally treated roundup ready GM soya. This resulted in the development of protein dystrophy of the kidneys and liver, which testifies to the reduction of adaptive opportunities of the organs. The most expressed declines from the norm were in the adrenal glands, which had considerable edema with discomplexation of the cells and dystrophy and atrophy of endocrine cells of all areas of the cortex, a criterion of functional exhaustion.*

Key words: *roundup GM soya, pigs, structure, liver, kidneys, adrenal glands*

Introduction

The first transgenic organisms were obtained in 1986 after successful field-testing of transgenic plants. Ten years later, the first genetically modified (GM) food products appeared in the United States: tomatoes with delayed ripening and roundup ready soy [Korytnyuk, Borysen Co., 2008a, b]. Two years later, GM corn, potatoes, zucchini, radish, rape, tobacco, cotton and others entered the market. For many countries, animal feed and food product for humans created from different GM crops became a hot topic. Concomitant use of biotechnology without a deep understanding of the consequences of its influence can lead to the most tragic consequences. The use of GMOs is among the least studied of questions, so it cannot be stopped and eliminated if necessary [Ermakov, 20]. The large-scale spread of GMOs and the potential introduction of foreign genetic material into the cells of plants, animals, humans can become a cause of irreversible pathological changes in the body of living creatures and lead to their extinction. To rule out the risks it is necessary to provide a comprehensive study of the biology of GM plants and the fundamentals of genome expression and regulation. At present, there is discussion about the production and use of GMOs that consists of almost opposite opinions. Thus, manufacturers and economists argue that GMOs are safe for consumption in certain doses based on the need to increase the production of food. Scientists, especially environmentalists, have proven that GMOs are harmful and require mandatory labeling of their presence in products approved for sale [Tutelyan et al., 2010; Tishko et al., 2010]. Continuous monitoring of each authorized GM product is required, as well as comprehensive and profound study of potential negative impacts not only of GMOs but also their metabolites, of which quality and quantity is difficult to predict [Zakrevskii, 2006; Kuznetsova, 2010; Seralini et al., 2012]. According to the authors, the use of biotechnology is a hidden, understudied global threat to the environment and nature in general. Unfortunately, there are have been instances when scientists who have proven that GMOs are harmful have been prosecuted by monopoly producers. This created conditions for the spread of biotechnology worldwide from the US and stopping this process became impossible [Ermakova, 2011; Dolaychuk, 2012]. This applies to the production and use of the Roundup ready soybeans. Producers and farmers interested in economic benefits today are so inspired by the use of the Roundup herbicide, that in circumstances of free access to it (including free access in Ukraine) it is difficult to exclude violations of technology use, especially with dosage. The postulate put forward by Ye.I.Makovskaja in 1968 has not lost its relevance today: "the more pesticide is effective - the more harmful it is for human".

In recent years in Ukraine, including in Podillja, there has been significantly increased production of Roundup ready GM soy [Babich, 2013]. It is known [Ermakova, 2006, 2008,

2009; Maligin, 2008; Tishko et al., 2010; Tutelyan, 2010; Matyukha, 2012; Malatesta et al., 2002, 2003; Veccio et al., 2003] that it affects the offspring of laboratory animals (rats, mice), including the structural and functional condition of the pancreas, gonads, liver and kidneys. Changes in the hormonal state of the organisms, the development of infertility and cancer have been noted. Given everything mentioned above, the purpose of this research was to study the functional state of the liver, kidneys and adrenal glands of pigs that had a long-term diet with Roundup ready GM soy.

Materials and Methods

We know from the literature [Zapadnyuk et al., 1983] that domestic pigs are considered one of the most efficient subjects to gain knowledge of the mechanisms of physiological and pathological processes in humans. Thus, to perform experimental research on the impact of Roundup ready GM soy, we used piglets of 2 months of age. The experimental group consisted of 4 pigs and 2 boars, which had a diet including (additionally to cereals) 15-20% of crude protein from thermally processed Roundup ready GM soybeans. A control group, with the same number of animals, was fed (additionally to cereals) 15-20% of crude protein from sunflower meal and grind peas. Research took place between 2011- 2013 and lasted 485 days.

Rations and conditions for both groups met the requirements and accepted standards of average growth. After the slaughter of animals, absolute and relative weights of their internal organs were determined. For histological studies, pieces of liver, kidneys and adrenal glands were taken, fixed in 10% neutral formalin solution, treated with alcohols and fixed in paraffin by generally accepted methods. Tissue sections were stained with hematoxylin eosin, and selectively with Van Gieson.

Results. Discussion

The external examination of the internal organs of experimental animals showed a slight increase in their absolute and relative weights (Table. 1).

Kidney tissue on the surface and in the cut is darker and more full-blooded than the control. Its consistency, as well as consistency of the liver, is somewhat softer. Other organs were without noticeable macroscopic abnormalities.

Microscopical analysis of the kidney detected an uneven plethora of glomeruli and stroma of the organ. There are single hemorrhagic extravasations, uneven stromal edema and widespread, almost with diffuse character, protein granular degeneration of the convoluted tubule epithelium with the presence of granular masses in the lumen (Fig. 1). There are also some regions of severe hydropic degeneration of epithelia resulting in colliquative necrosis. Epithelial cells of convoluted tubules are significantly increased in volume, swollen, and contain nuclei with different numbers of chromatin or have no nucleus (Fig. 2). Sometimes tubulorrhexis is present - its collapse is due to considerable swelling and bleeding in surrounding stroma and rupture of the tubule wall. Also present are small size tubules without lumen, which are intensely lined with eosinophilic epithelial cells. Among these, cells with two nuclei are found frequently. This tissue is limited by fibrous connective tissue that is a sign of regeneration and atrophy.

Table 1. Absolute and relative weight of domestic pigs inner organs after long-term diet with Roundup Ready GM soybeans.

Measure, kg	Control group		Experimental group		± relatively to control
live weight	203.3	% relatively to live weight	221.7	% relatively to live weight	
Liver	2.4	1.18	2.790	1.26	+0.08
Kidney	0.407	0.20	0.630	0.28	+0.08
Hart and lungs	1.91	0.94	2.15	0.97	+0.03

Spleen	0.283	0.14	0.397	0.18	+0.04

Microscopic study of liver architectonics didn't find violations of its structure. Orientation of hepatocytes in plates was preserved. However, a significant swelling of portal tracts with increase in number and uneven expansion of lumen of lymphatic clefts and vessels were noted (Fig. 3). Inside particles an expansion of the space of Disse occurs. Blood supply of sinusoids and the central vein was not different from control animals. Sometimes a moderate swelling and proliferation of Kupffer cells and endothelial cells were revealed. Parenchymal cells are characterized by grit cytoplasm, which appears in most hepatocytes. Their nuclei are polymorphic with different content of chromatin. Sometimes among these altered cells are small clusters of necrotizing hepatocytes covering 1-4 cells. The contours of such cells are not clear or are absent. Some hepatocytes are reduced in volume, with homogeneous hypereosinophilicum cytoplasm and deformed hyperchromic or fragmented nuclei, which is typical of apoptosis. Around foci of necrosis and apoptosis a minor infiltration of mononuclear cells are identified (Fig. 4). Hepatocytes, which are located near the foci of necrosis and apoptosis, often have signs of intracellular regeneration and adaptation that is manifested as hypertrophy and polyploidy. They have hyperchromic nuclei with two or more nucleoli. Groups of hepatocytes that have monomorphic structure were found close to each other; they are the same size, have normal cytoplasm and small normochromic nuclei with one nucleolus. This indicates a loss of structural heterogeneity, that in our view is a sign of stress and reduce of functional adaptive reserves of liver.

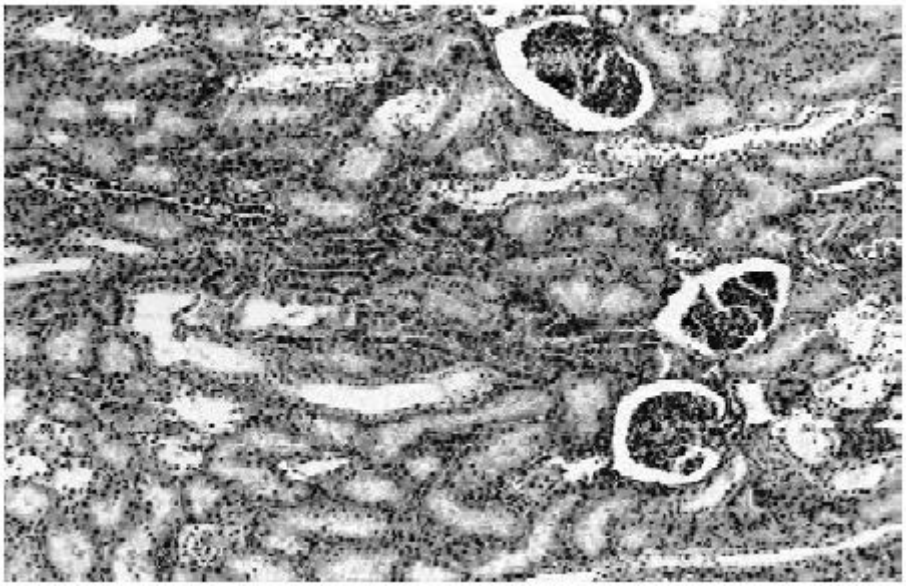

Fig. 1. Kidney. Grainy dystrophy of the convoluted tubule epithelium with the presence of eosinophilic masses in the lumen. Hematoxylin-eosin. x100.

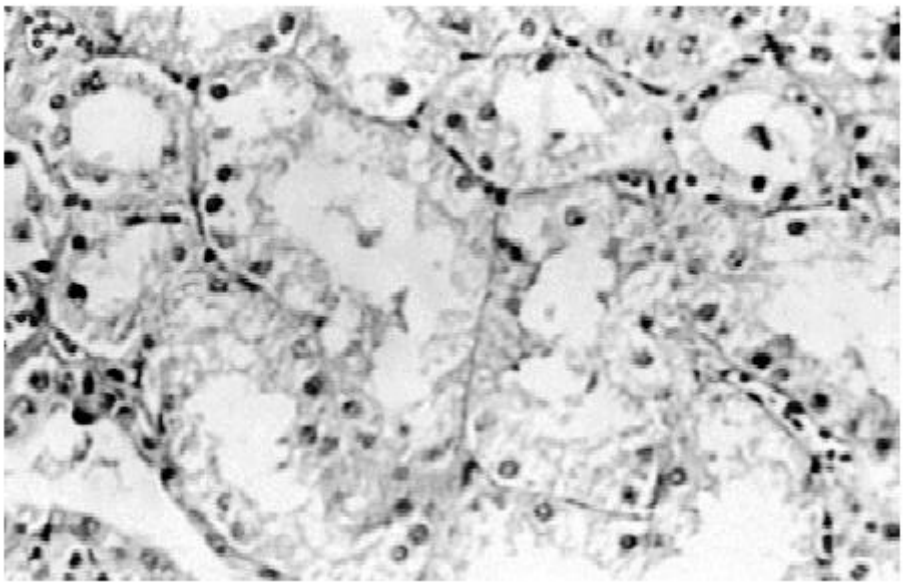

Fig. 2. Kidney. Hydropic degeneration and necrosis of the convoluted tubule epithelium. Hematoxylin-eosin. h400.

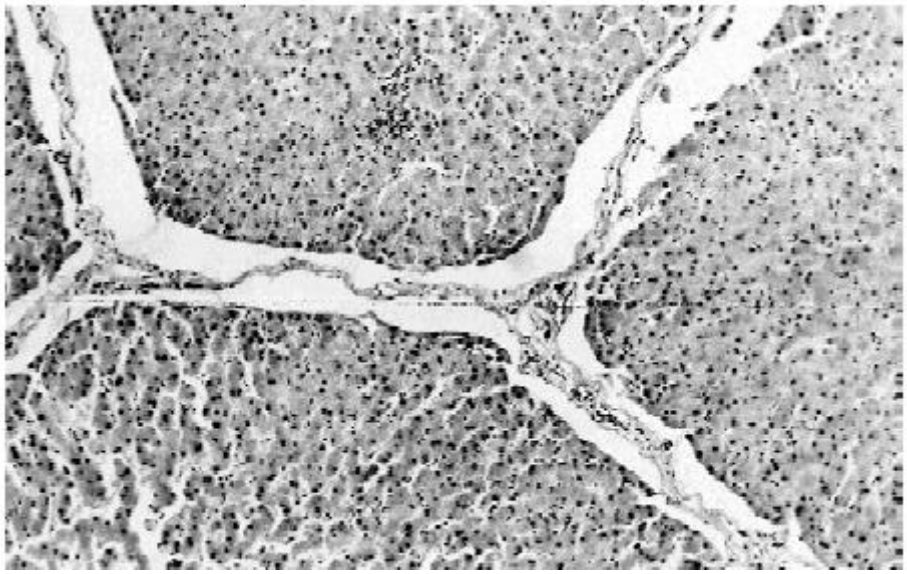

Fig. 3. Liver. Expansion of portal way because of significant edema. Anisocytosis and aniso nucleosis of hepatocytes. Hematoxylin-eosin. x100.

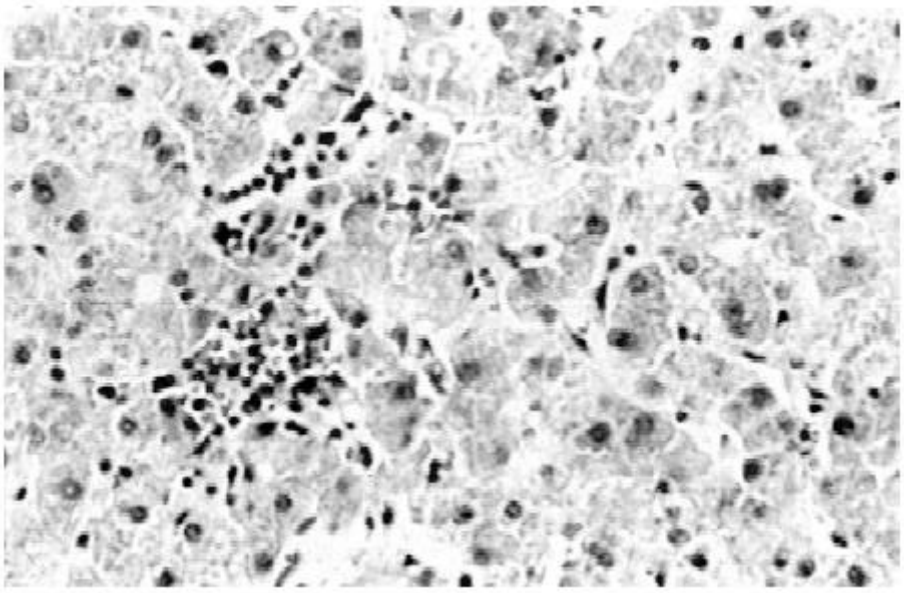

Fig. 4. Liver. Focal infiltration of mononuclear cells against a background of granular dystrophy of hepatocytes. Hematoxylin-eosin. x400.

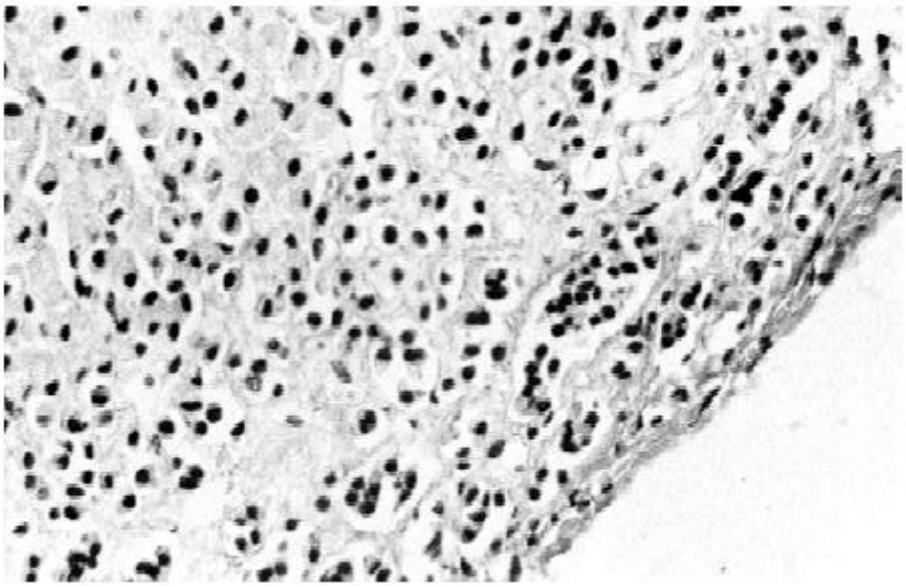

Fig. 5 Adrenal glands. Thinning and moderate sclerosis of glomerular zone in cortical layer. Hematoxylin-eosin. x100.

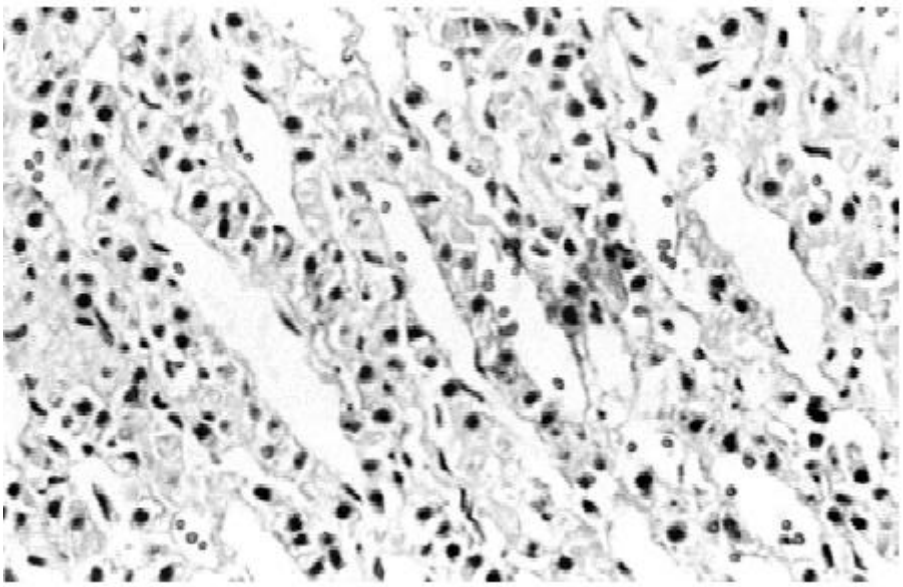

Fig. 6. Adrenal gland. Endocrinocytes of zona fasciculate bands are in close contact with each other, have a moderately granular vacuolated cytoplasm. Hematoxylin-eosin. x400

Particularly noteworthy are the microscopic changes in the adrenal glands because their regulatory changes can influence other organs. The capsule of the organ is unevenly thickened due to edema and increased collagen fibers. Along it, thin branches of collagen fibers in the glomerular zone of the adrenal cortex are observed. With regard to the glomerular zone, its uneven thickness draws attention. In some places, there are scanty small groups of endocrinocytes cells with hyperchromic nuclei and cytoplasm of different eosinophily. Among them are many cells with two nuclei. Groups limited by broad layers of connective tissue (Fig. 5). Such changes are characteristic of productive mineralocorticoids atrophy of glomerular zone which is offset by the increased proliferation of certain endocrinocytes.

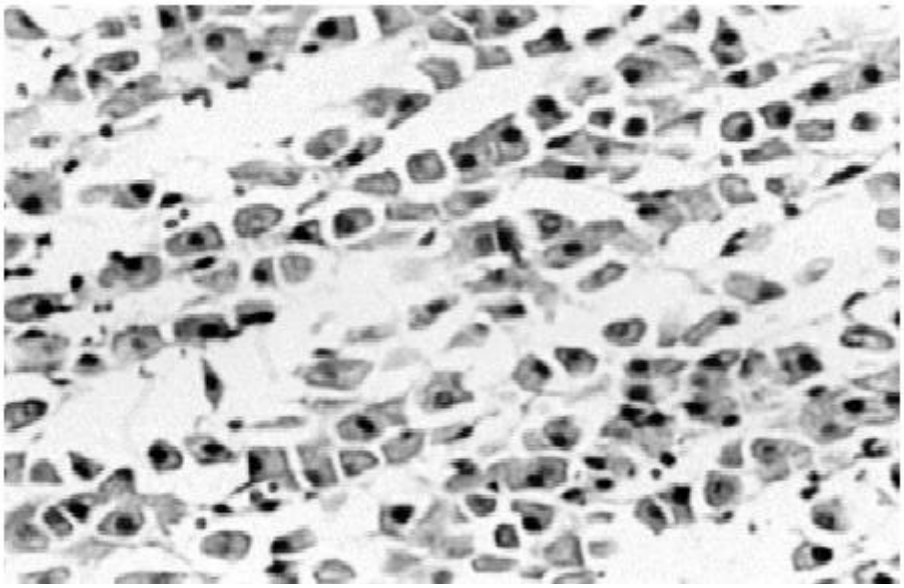

Fig. 7. Adrenal gland. Swelling and discomplexation of endocrinocytes with homogeneous cytoplasm. Hematoxylin-eosin. x400.

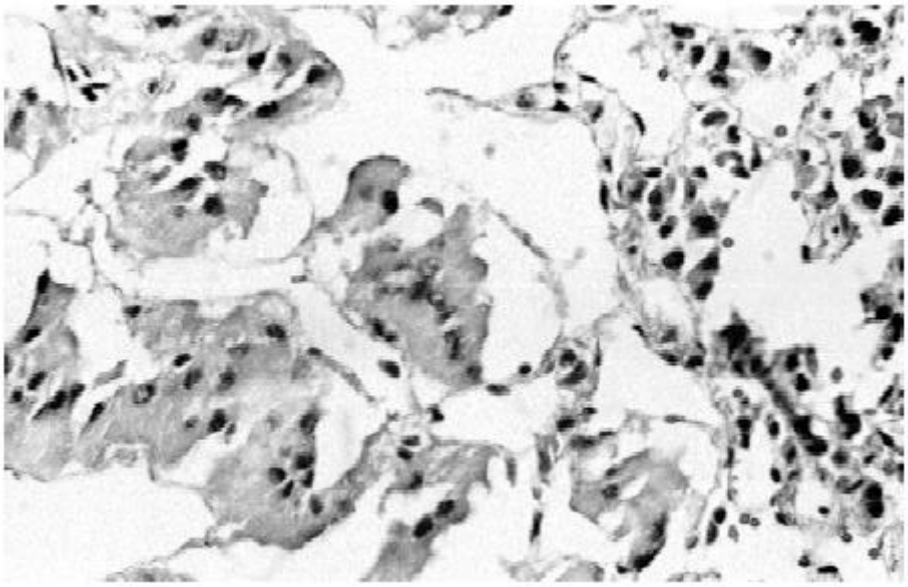

Fig. 8. Adrenal gland. Significant expansion of microcirculation vascular lumen by transparent liquid in medulla. Hematoxylin-eosin. x400.

In the zona fasciculate, there is a significant extension of transparent liquid of lumen of the direct sinusoidal capillary lined with fenestrated endothelium. This leads to the weakening of cell-cell contacts and cell decomplexation in bands through the accumulation of oedematous fluid. In some places of sinusoid lumens, clusters of red blood cells characteristic for hyperemia were found. Regarding bands of endocrinocytes in the zona fasciculate, their structure is different. Along with areas where endocrinocytes have moderately enlightened or granular cytoplasm are in tight contact with each other (Fig. 6), there are areas where endocrinocytes are different in sizes, have homogeneous and increased eosinophilic cytoplasm, have hyperchromic nuclei, and have absent contacts with each other (Fig. 7). In areas of significant edema, accumulations of necrotic cells without nuclei were found. We evaluated these changes as signs of profound atrophy, necroapoptosis induced by pathogens, and functional signs of exhaustion. Along with these changes in the zona fasciculate, centers of moderate sclerosis with presence of unextended mononuclear infiltrates were found. In the reticular zone and medulla of the adrenal glands, degenerative changes of endocrinocytes and significant uneven expansion of the microcirculation vascular lumen by transparent liquid, which is typical for swelling, were found (Fig. 8).

Conclusions and recommendations for further studies

1. Long-term feeding of pigs by Roundup ready GM soybean causes swelling and degenerative changes in the kidneys and liver, which indicate a decrease of the reserve adaptive capacity of organs.

2. Significant abnormalities in the structure of the adrenal glands were found, including degeneration and atrophy in all areas of the cortical layer and swelling with decomplexation of endocrinocytes, which can cause a functional depletion of the organ.

3. Study results should be considered in the consumer regulation of Roundup ready GM soybean. Morphological changes revealed in the adrenal glands can serve as a basis for identifying new areas of research related to the regulatory mechanisms of endocrine glands in terms of Roundup ready GM soy consumption.

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